

University of Groningen

Integrating biomarkers to predict renal and cardiovascular drug efficacy

Schievink, Bauke

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

2016

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Schievink, B. (2016). *Integrating biomarkers to predict renal and cardiovascular drug efficacy: PRE score applications from drug registration to personalized medicine*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Chapter 4

Prediction of the effect of atrasentan on renal and heart failure outcomes based on short-term changes in multiple risk markers

Bauke Schievink

Dick de Zeeuw

Paul A Smink

Dennis Andress

John J Brennan

Blai Coll

Ricardo Correa-Rotter

Fan Fan Hou

Donald Kohan

Dalane W. Kitzman

Hirofumi Makino

Hans-Henrik Parving,

Vlado Perkovic,

Giuseppe Remuzzi

Sheldon Tobe

Robert Toto

Jarno Hoekman

Hiddo Lambers Heerspink



Abstract

Background: A recent phase II clinical trial (RADAR/JAPAN) showed that the endothelin A receptor antagonist atrasentan lowers albuminuria, blood pressure, cholesterol, hemoglobin, and increases body weight in patients with type 2 diabetes and nephropathy. We previously developed an algorithm, the PRE score, which translates short-term drug effects into predictions of long-term effects on clinical outcomes.

Design: We used the PRE score on data from the RADAR/JAPAN study to predict the effect of atrasentan on renal and heart failure outcomes.

Methods: We performed a post-hoc analysis of the RADAR/JAPAN randomized clinical trials in which 211 patients with type-2 diabetes and nephropathy were randomly assigned to atrasentan 0.75mg/day, 1.25mg/day, or placebo. A PRE score was developed in a background set of completed clinical trials using multivariate Cox models. The score was applied to baseline and week-12 risk marker levels of RADAR/JAPAN participants, to predict atrasentan effects on clinical outcomes. Outcomes were defined as doubling serum creatinine or end-stage renal disease and hospitalization for heart failure.

Results: The PRE score predicted renal risk changes of -23% and -30% for atrasentan 0.75 and 1.25 mg/d, respectively. PRE scores also predicted a small non-significant increase in heart failure risk for atrasentan 0.75 and 1.25 mg/d (+2% vs. +7%). Selecting patients with >30% albuminuria response from baseline (responders) improved renal outcome to almost 50% risk reduction, whereas non-responders showed no renal benefit.

Conclusions: Based on the RADAR/JAPAN study, with short-term changes in risk markers, atrasentan is expected to decrease renal risk without increased risk of heart failure. Within this population albuminuria responders appear to contribute to the predicted improvements, whereas non-responders showed no benefit. The ongoing hard outcome trial (SONAR) in type 2 diabetic patients with >30% albuminuria response to atrasentan will allow us to assess the validity of these predictions.

Introduction

Despite the availability of existing proven therapies to slow progression of kidney disease, diabetic nephropathy remains associated with a high risk of end-stage renal disease (ESRD) (1,2). Endothelin-A receptor antagonists (ERA) have been proposed as an addition to blockade of the renin-angiotensin-aldosterone system (RAAS) to delay progression of kidney disease (3-6). Clinical trials with the ERAs avosentan, darusentan and sitaxsentan have shown reductions in albuminuria (7-9). The recent Reducing Residual Albuminuria in Subjects With Diabetes and Nephropathy With AtRasentan trial and an identical trial in Japan (RADAR/JAPAN trials) showed that the addition of the ERA atrasentan in doses of 0.75 and 1.25 mg/day on top of angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) therapy results in approximately 40% reduction in albuminuria in patients with type 2 diabetes and nephropathy (10). These data suggest that ERAs may confer renoprotective effects, although this has not been confirmed in a long-term hard outcome trial.

In the RADAR/JAPAN trials atrasentan not only decreased albuminuria, but also blood pressure and LDL cholesterol (10). These additional effects may enhance the beneficial effect of atrasentan. In contrast, atrasentan dose-dependently increased body weight reflecting sodium retention, which induced edema and may increase the risk of congestive heart failure (CHF) as seen with avosentan. The sodium-retaining effect may thus negatively influence the benefit-risk ratio of ERAs, and has indeed led to early discontinuation of other clinical trials with ERAs (11,12). Because ERAs have effects on multiple renal or cardiovascular risk markers, known risk markers should ideally be measured and integrated in order to reliably predict the long-term effect of atrasentan on clinical outcomes, as opposed to using a single marker (such as albuminuria) alone. We recently developed and validated an algorithm, the multiple Parameter Response Efficacy (PRE) score, that predicts long-term risk of clinical outcomes based on short-term effects of drugs on multiple risk markers (13,14). The PRE score was developed because single drugs have effects on multiple renal/cardiovascular risk markers, and each of these effects may alter the ultimate renal/cardiovascular outcome (15). The aim of the current study is to apply the PRE score to data from the RADAR/JAPAN trials to predict the potential long-

term effect of the ERA atrasentan on renal and heart failure outcomes. In addition, the benefit-risk ratio for the 0.75 and 1.25 mg/day atrasentan doses was established.

Methods

Principles of the PRE score model

We predicted the effect of atrasentan on renal and heart failure outcomes by using the PRE score. This score translates the effect of short-term drug-induced changes in multiple risk markers to long term outcomes as previously described (13,14). In brief, first we established the relationship between risk markers and clinical outcomes in a background population of completed clinical trials with similar patient characteristics as the RADAR/JAPAN trials (NCT01356849 and NCT01424319). We matched our background population to be able to calculate risk marker outcome relationships that are representative of patients included in the RADAR study. The background population was selected from completed clinical trials including the Reduction of Endpoints in NIDDM with the Angiotensin II Receptor Antagonist Losartan (RENAAL), Irbesartan Diabetic Nephropathy Trial (IDNT), the Aliskiren Trial In Type 2 Diabetes Using Cardiorenal Endpoints (ALTITUDE) and The Avasentan on Time to Doubling of Serum Creatinine, End Stage Renal Disease or Death (ASCEND) trial. Because patients in the RADAR/JAPAN trials used maximum ACEi or ARB, we selected all patients from the background datasets that used ACEIs or ARBs for at least six months.

Secondly, the calculated risk marker-outcome relationships, established in the background population, with a median follow-up of almost 3 years, were applied to the baseline and week-12 risk marker measurements in the atrasentan 0.75 mg/day and 1.25 mg/day treatment arm of the RADAR/JAPAN trials to predict individual patients' 3-year renal (defined as a composite of sustained doubling of serum creatinine or ESRD) or heart failure risk (defined as hospitalization due to CHF) at both time points. These endpoints were recorded in the background database and were pre-specified and adjudicated by an independent endpoint committee using rigorous and standardized definitions. Thirdly, we calculated the mean of the difference in the predicted risk at baseline and week 12, adjusted for the mean of the difference in predicted risk in the placebo arm. This represented the PRE score and

indicated the 3-year renal or heart failure risk change conferred by atrasentan. In summary, the background database was used to establish the association between multiple risk markers and clinical outcomes using a population which was similar to the RADAR/JAPAN population. Based on the risk marker outcome associations in the background database we predicted renal/heart failure risk change by considering the changes in multiple risk markers as observed in the RADAR/JAPAN trials. The model assumes that the predictive ability of short-term risk marker changes are independent of the drug that induces the change. As such the model is applicable to different interventions as long as all relevant risk marker changes are measured and included.

We also applied the risk score to a subset of responders (>30% reduction in albuminuria) and a subset of non-responders (<30% albuminuria reduction) in the RADAR/JAPAN trials to assess whether enriching the patient population (only including responders after a run-in period) would improve the benefit-risk profile of atrasentan.

Risk marker selection in the RADAR/JAPAN trials

All parameters that were measured in the intention-to-treat population of the RADAR/JAPAN trials and were previously identified as risk markers for cardiovascular or renal clinical outcomes were used for analysis: urine albumin-to-creatinine ratio (albuminuria), body weight, systolic blood pressure (SBP), hemoglobin (Hb), albumin, calcium, HbA1c, LDL cholesterol, HDL cholesterol, serum potassium, phosphate and uric acid. Albuminuria was measured from the geometrical mean of 3 first morning void urine samples in a central laboratory in the United States (Quest Diagnostics Clinical Trials, Valencia, CA, USA) or Japan (BML, Inc., Saitama, Japan). We included all available risk markers to capture all measured short-term effects of atrasentan. PRE scores were calculated for subjects in RADAR/JAPAN in whom all risk markers were measured at baseline and follow-up.

Changes in body weight were used as a proxy for the sodium-retaining effects of atrasentan, which likely represents a different heart failure risk than other forms of body weight change (such as fat deposition). Because no data on fluid-specific body weight changes was present in the RENAAL, IDNT and ALTITUDE trial, we included data from the ASCEND trial in which patients were treated with the ERA avosentan.

From the ASCEND data we could assess the direct association between ERA-induced body weight changes and renal and heart failure outcomes.

Statistical analysis

Data are presented as means and standard deviation or counts and percentages. Risk markers were handled as continuous variables. Changes in risk marker levels at week 12 between treatment arms were tested with ANCOVA adjusted for baseline values and Tukey post-hoc tests for pairwise comparisons. A Cox proportional hazards model was used to estimate the coefficients and hazard ratios associated with each risk marker for the first recorded renal or heart failure event. Non-normally distributed data was log-transformed. The regression coefficients for each risk marker were taken and used as weights for the risk equation for renal and heart failure outcomes. The risk equations for renal and heart failure outcomes were applied to the risk markers observed in the RADAR/JAPAN trials at baseline and week 12 to calculate renal and heart failure risk at both time points. The mean difference in risk between the two time points, after subtracting the mean risk difference between both time points in the placebo arm, represented the PRE score for each outcome.

We performed additional analyses with a simulated range of atrasentan-induced albuminuria changes and body weight changes. We simulated the albuminuria response because it is an important predictor for renal outcomes (16). We simulated body weight change because it is a strong predictor for heart failure induced by fluid retention (17). Simulations were performed by shifting the distribution of the albuminuria and body weight response. This sensitivity analysis was performed to take into account that atrasentan may have different effects on these risk markers in the long-term hard outcome trial.

A two-tailed p value of <0.05 indicated statistical significance. All statistical analyses were conducted with R version 3.0.1 (R Project for Statistical Computing, www.r-project.org).

Results

The characteristics of all subjects included in the calculations are presented in Table 1. Out of the 211 subjects in the RADAR/JAPAN dataset, 164 (78%) had a complete risk marker profile at baseline and follow-up. Of the 164 subjects derived from the RADAR/JAPAN dataset, 119 were randomized to atrasentan treatment (59 patients 0.75mg/d and 60 patients 1.25mg/d). A total of 63 (52.9%) patients were classified as responders (27 patients 0.75mg/d, 36 patients 1.25mg/d), while 56 (47.1%) patients were classified as non-responders (32 patients 0.75mg/d, 24 patients 1.25mg/d).

Table 1. Baseline characteristics of subjects in the background and RADAR/JAPAN dataset. Numbers indicate mean (SD), unless otherwise specified.

	Background (N=2466)	RADAR/JAPAN Complete cases (N=164)
Age, years	61 (9)	65 (9)
Males N (%)	1630 (66)	124 (76)
Race N (%)		
White	1328 (54)	65 (40)
Black	224 (9)	21 (13)
Asian	631 (26)	74 (45)
Other	283 (11)	4 (2)
Systolic blood pressure, mmHg	142 (18)	137 (14)
Weight, kg	83 (20)	86 (21)
eGFR (ml/min/1.73m ²)	41.0 (13)	49.3 (14)
HbA1c, %	8.1 (1.8)	7.4 (1.4)
Hb, (g/dl)	12.5 (1.9)	12.9 (1.7)
LDL cholesterol, mg/dl	114 (43)	93 (35)
HDL cholesterol, mg/dl	45 (14)	47 (13)
Serum K ⁺ , mEq/l	4.7 (0.6)	4.5 (0.5)
Phosphate, mg/dl	3.9 (0.8)	3.6 (0.6)
Serum albumin, g/dl	4.0 (0.4)	4.0 (0.4)
Calcium, mg/dl	9.2 (0.5)	9.2 (0.5)
Uric acid, mg/dl	7.1 (1.8)	7.7 (1.8)
UACR (median mg/g, IQR)	1026 (491-2108)	863 (469-1544.0)

Abbreviations: SBP, systolic blood pressure; UACR, urinary-albumin-to-creatinine ratio (albuminuria); Hb, hemoglobin; K⁺, potassium; IQR, interquartile range.

A total of 2466 cases were used to calculate risk marker-outcome relationships in the background dataset. In the background dataset 331 patients reached a renal event and 130 patients a hospitalization for heart failure event.

Short term risk marker changes

Figure 1 shows changes in risk markers after treatment with placebo, atrasentan 0.75 mg/d, or atrasentan 1.25 mg/d for the overall RADAR/JAPAN population. As previously shown, atrasentan at doses of 0.75 mg/d and 1.25 mg/d induced a significant reduction in urinary albumin excretion of approximately 40%. Atrasentan also decreased blood pressure, hemoglobin, LDL cholesterol, and increased body weight (Figure 1).

By restricting the population to responders (>30% albuminuria reduction), we found a mean decrease in albuminuria of approximately 60% for both the 0.75 mg/d and 1.25 mg/d atrasentan dose, respectively. Non-responders had no significant change in albuminuria (Supplemental Figure S1). Changes in risk markers other than albuminuria were similar between responders and non-responders (Supplemental Figure S1), suggesting that responses in other risk markers were independent of the albuminuria response.

Predicted treatment effect

Based on the albuminuria-lowering effect of atrasentan 0.75 mg/d and 1.25 mg/day alone, we predict a relative risk reduction for renal events of 31% and 37% respectively. However, atrasentan induced short-term changes in body weight and hemoglobin could imply an increase in renal risk (Figure 2). The PRE score, that integrates the effect of atrasentan on all risk markers, indicated a relative renal risk change of -23% (95% CI: -47% to +1%) for the 0.75 mg/d dose and -30% (-55% to -6%) for the 1.25 mg/d dose (Figure 2). For the heart failure endpoint, the PRE score indicated a slightly higher risk increase for the 1.25 mg/d dose (+7%; -13% to +27%) compared to the 0.75 mg/d dose (+2%; -16% to +20%) (Figure 3). Changes in body weight and Hb were the main contributors to the adverse effect prediction for heart failure. Results were similar when missing values in risk markers of RADAR/JAPAN subjects were imputed.

In the RADAR/JAPAN trials, we observed a large variability in albuminuria response to atrasentan (5th to 95th percentile -74.8% to +48.4%). When we restricted

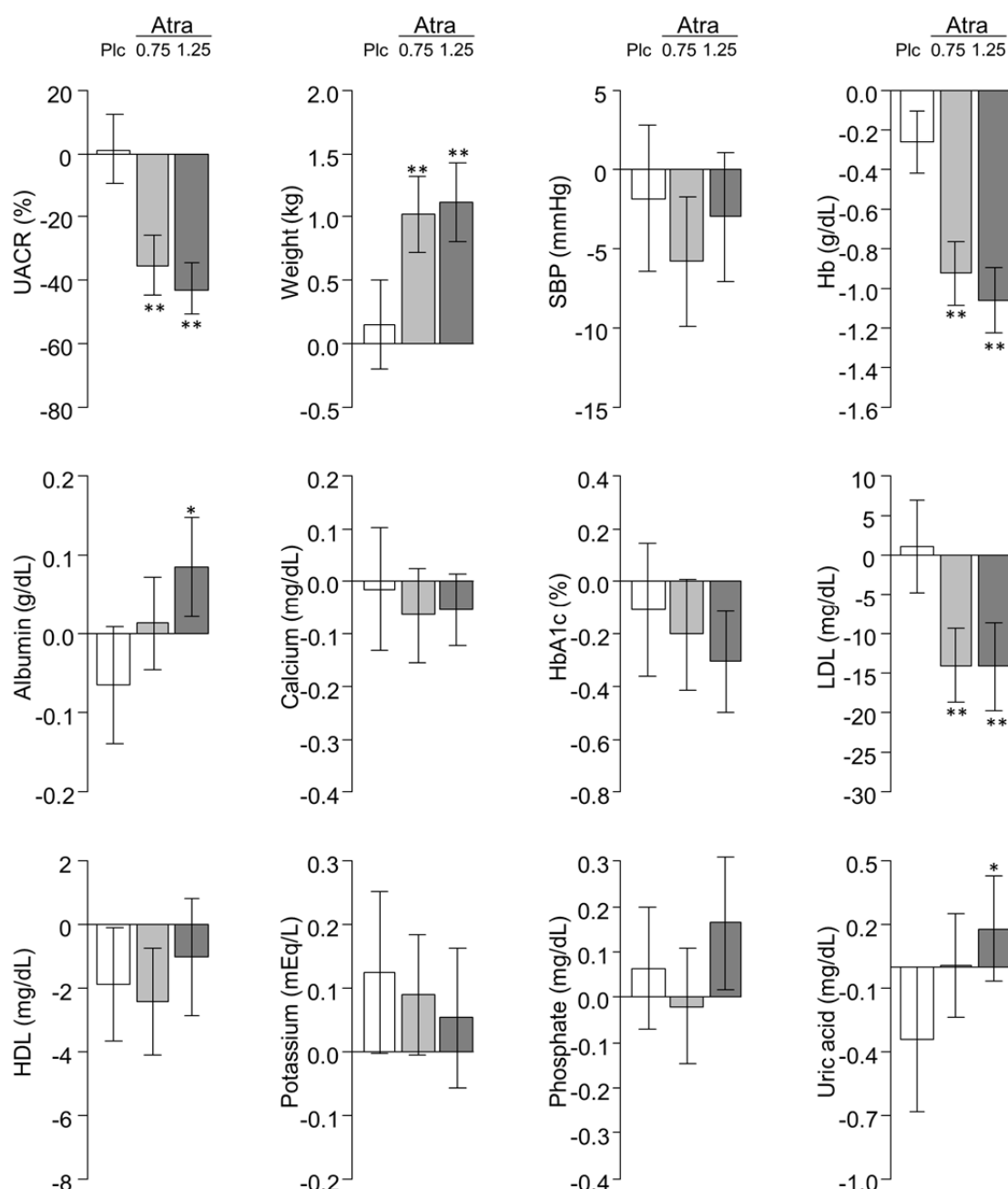


Figure 1. Overview of the changes (mean + 95% CI) in risk markers in RADAR/JAPAN in the placebo, atrasentan 0.75 mg and atrasentan 1.25 mg group after 12 weeks of follow-up. Results are shown for the total population. * $P < 0.05$, ** $P < 0.001$ versus placebo. Abbreviations: SBP, systolic blood pressure, Hb, hemoglobin.

the population to only albuminuria responders ($>30\%$ albuminuria reduction), the estimated renal risk reduction conferred by atrasentan 0.75 mg/d was -47% (-71% to -23%), with similar results in the 1.25 mg/d group (-47% , -71% to -22% ; Figure 2). However, the PRE score for heart failure in the responder group predicted lower risk for the 0.75mg/d dose (-9% , -29% to $+11\%$) than with the 1.25mg/d dose ($+5\%$, -19% to $+29\%$; Figure 3). For non-responders, the PRE score predicted no renal

benefit for both doses: +4% (-22% to +31%) and +4% (-31% to +39%; Figure 2) for 0.75 mg/d and 1.25 mg/d respectively. For heart failure the estimates were +12% (-10% to +33%) and +10% (-17% to +36%; Figure 3), respectively. PRE score predictions were similar after imputation of missing risk marker values at baseline and week 12 in the RADAR/JAPAN trials (Supplementary table S1).

We finally assessed whether we could identify baseline determinants of a favorable PRE score. In univariate and multivariate analyses, none of the baseline risk markers showed an association with the PRE scores of each individual.

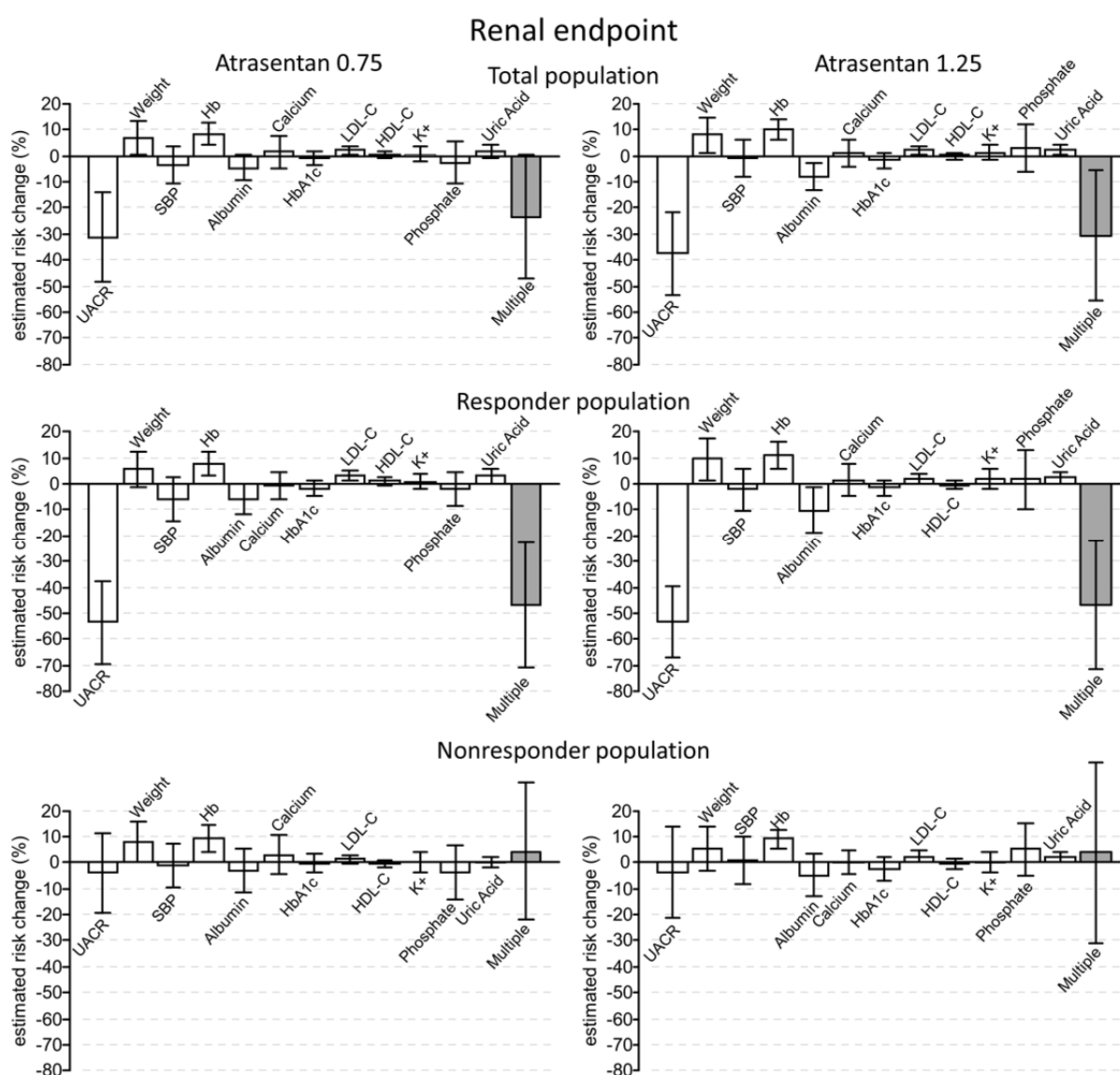


Figure 2. Predicted risk change for renal outcomes for the total, responder and non-responder population based on changes in single risk markers and the integrated effect of all risk markers. Bars indicate percentage mean change in relative risk induced by treatment corrected for placebo + 95% confidence interval (CI).

Discussion

Using a multiple parameter response efficacy (PRE) score and the short-term response data to atrasentan, we predicted that long-term treatment with atrasentan on top of ACEi or ARB therapy would reduce renal risk without a significant increase in heart failure risk. However, confidence intervals for the heart failure outcome were wide, therefore leaving a certain degree of uncertainty. The albuminuria response is by far the most important contributor to the potential renoprotective effect. When the calculations were restricted to a responder subset defined as a >30% decrease in albuminuria, the PRE score showed a significant further reduction in predicted renal risk with no apparent dose difference. Similar to the overall population, the risk of heart failure among albuminuria responders was smaller with the 0.75 compared to the 1.25 mg/d dose of atrasentan, suggesting that renoprotective effects without significant risk of heart failure may be achieved with atrasentan 0.75 mg/d in this population.

Proper dose selection is a key design element for any clinical trial and particularly for drugs with a narrow therapeutic index such as ERAs. The maximum recommended therapeutic dose is determined from pharmaceutical dose-response trials and represents the dose of the pharmaceutical agent with the optimal benefit-risk ratio. Knowledge about the dose-response relation of a drug is important because it provides information on the dose beyond which no additional benefit is expected or side effects become unacceptable (18). Past experience teaches us that selecting the optimal dose in clinical studies for ERAs is problematic. A phase 2 study with the ERA avosentan showed dose-dependent reductions in albuminuria with a maximum anti-albuminuric dose of 10 mg/day (7). Avosentan at doses beyond 10 mg/day did not further decrease albuminuria and caused a dose-dependent increase in body weight and fluid retention. Despite these findings avosentan at doses of 25 mg/day and 50 mg/day was tested in a hard outcome trial, that was terminated early due to excess heart failure and mortality in the avosentan treatment arms (11). This example illustrates the importance of carefully conducted dose-ranging studies in early stages of drug development. If validated in a full-scale trial, the PRE score can aid in the selection of the optimal dose and thereby improve clinical trial design, because it takes all known risk markers into account and

integrates them into a composite risk score. Our analysis shows that among albuminuria responders, atrasentan 0.75 mg/d and 1.25 mg/d have a similar predicted protective effect on renal outcomes. We predicted a slightly higher risk with the 1.25 vs. 0.75 mg/day dose of atrasentan on heart failure but the wide confidence intervals around the predicted effect preclude definitive conclusions regarding dose selection. In combination with results of additional pharmacokinetic and pharmacodynamic analyses, atrasentan 0.75 mg/d is selected for use in a long-term hard outcome trial.

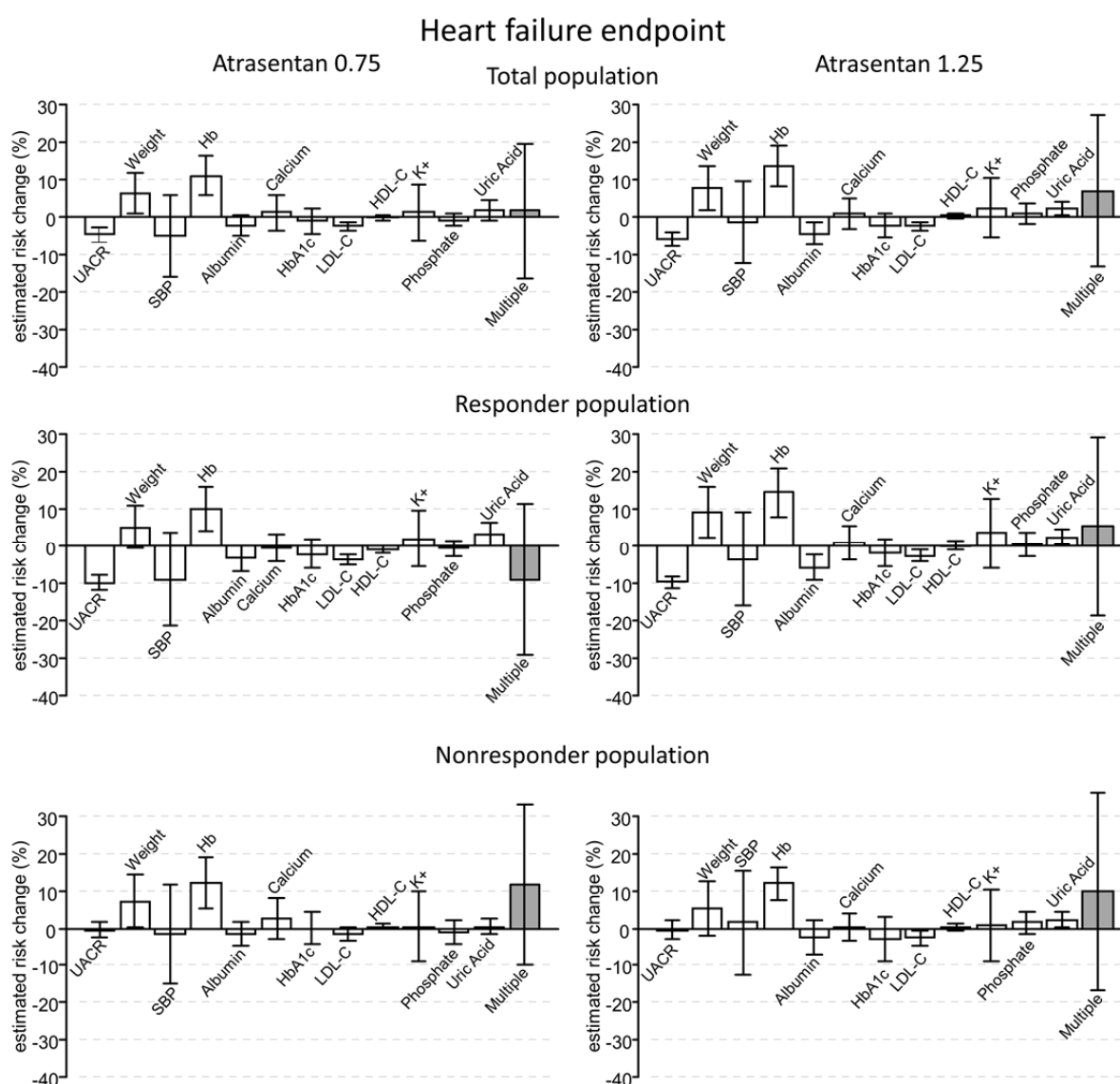


Figure 3. Predicted risk change for heart failure for the total, responder and non-responder population based on changes in single risk markers and the integrated effect of all risk markers. Bars indicate percentage mean change in relative risk induced by treatment corrected for placebo + 95% confidence interval (CI).

Another important aspect to consider when designing a new clinical trial is the response to treatment and enrichment of the trial population with treatment responders. Our results indicate that the reduction in albuminuria was the most important contributor to the renoprotective prediction of atrasentan. In fact, the current study predicted that patients with >30% albuminuria reduction (responders) would have a clear protective benefit whereas those with less albuminuria reduction (non-responders) showed no predicted benefit. These findings suggest that randomizing the non-responders in a hard outcome trial would not only increase the total number of patients needed to be enrolled in such a trial, but would also expose these patients to long-term atrasentan treatment, potentially without any likelihood of benefit. A hard outcome study enriching the clinical trial population with albuminuria responders, by exposing all patients to atrasentan during an 'enrichment' period prior to randomization could enhance the likelihood of detecting a renoprotective treatment effect. However, this requires validation in a prospective randomized controlled trial.

Another advantage of an enrichment period is that patients who experience side effects, such as fluid retention, can be identified during this period and can be excluded from randomization. Selection of patients for a clinical trial based on the response to the drug thus maximizes the beneficial effect and minimizes exposure of the intervention to patients in whom it may be harmful (19). This approach mimics daily practice since in daily clinical care the dose and type of drugs are adjusted based on the response of the patients to the drug. However due to the stringent selection criteria of the enriched randomized population, the generalizability of the results to a broader population with type 2 diabetes and nephropathy is limited when conducting an enrichment trial. Such an enrichment design is applied in the Study Of Diabetic Nephropathy With Atrasentan (SONAR, Clinical Trial identifier: NCT01858532) hard outcome trial with atrasentan. SONAR will enroll patients with similar characteristics to those in RADAR/JAPAN (i.e. type 2 diabetes and nephropathy). Eligible patients will proceed to a 6-week enrichment period. The aim of this enrichment period is to determine albuminuria response as well as safety. After completion of the 6-week enrichment period, patients with a response in albuminuria (>30% reduction) and without unacceptable rise in body weight (<3kg) or BNP (<300pg/ml) will be randomly assigned to long-term treatment with atrasentan or placebo. The SONAR trial will provide a more clear answer as to whether

atrasentan confers renoprotection and whether the PRE score algorithm was actually accurate in predicting the clinical benefits of ERA.

The reduction in albuminuria was an important driver of the predicted renal risk reduction. Prior trials with dual RAAS blockade showed a reduction in albuminuria and blood pressure but did not observe a renal risk reduction. However, in these trials off-target effects such as hyperkalemia, hypotension and a decrease in hemoglobin were observed which may offset the beneficial effect of albuminuria reduction. The PRE score integrates these multiple effects and translates them into long-term risk change. In RADAR/JAPAN, we noted that body weight and hemoglobin are the main contributors to increased renal and cardiovascular risk, whereas other risk markers had negligible effects. We previously applied the PRE score to a trial in which patients with type 2 diabetes and nephropathy were treated with aliskiren, and predicted that aliskiren treatment in the ALTITUDE trial would not result in the expected cardio-renal risk reduction calculated based on albuminuria reduction alone, due to off-target effects (hyperkalemia, hypotension) that negatively influenced the ultimate cardio-renal outcome (13). This was later confirmed by the early termination of the ALTITUDE trial. We note that based on this study no inferences can be made as to whether albuminuria is a valid target for renoprotective therapies. The SONAR trial with atrasentan, in which patients are randomized based on their albuminuria response, will provide more insight into this question.

The PRE score may not only have implications for drug development or drug regulation but also for predicting the ultimate treatment effect on clinical outcomes in individual patient care. In current practice drug efficacy is monitored based on single risk markers, such as blood pressure for an antihypertensive drug. However, the PRE score may offer the physician and patient a better tool to estimate the overall predicted drug effect (15). In a recent study we showed that, on an individual level, the PRE score indeed provides a better prediction of who will benefit from RAAS treatment compared to using single markers alone (20).

We could not predict the calculated PRE scores based on baseline values of risk markers. Similarly, none of the risk markers at baseline was able to predict the albuminuria-lowering or weight-increasing effect of atrasentan. Therefore, it is not possible to predict before exposure who will benefit from treatment using traditional recorded physical and clinical chemistry parameters. The development and implementation of novel tools, such as genomics, proteomics, or metabolomics, may

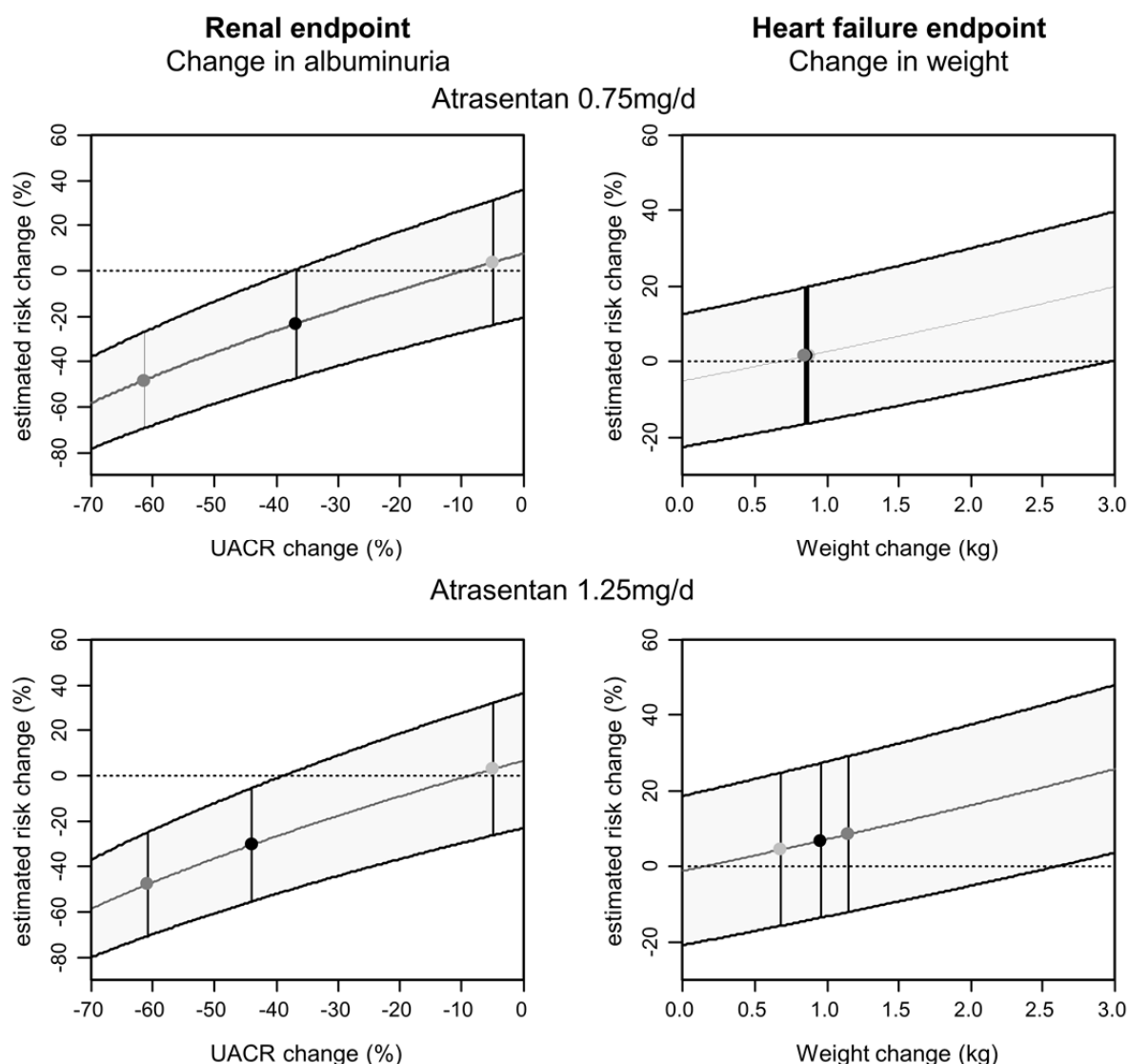


Figure 4. Simulated UACR and weight changes and the effect on renal and heart failure outcomes, respectively. The shaded area reflects the 95% CI for the simulated UACR/weight responses. The black dot indicates changes observed in the total RADAR/JAPAN population + 95% CI. The dark and light grey dots represent changes observed in the albuminuria responder and non-responder population respectively. Predicted risk was calculated with risk marker changes from the total population + simulated values for either albuminuria or body weight.

lead in the near future to more detailed phenotyping and may provide new insights and knowledge about individual determinants of treatment response.

Some aspects of our model should be considered. First, we included all measured cardiorenal risk markers in the model to capture all potential measured effects of atrasentan. We note that some of the included risk markers may not be causally related to renal or heart failure outcomes, despite their association with outcome. However, even in the case that some of the included risk markers are not causally related to outcome, they may still be representative of the underlying

disease process and, as such, may accurately predict outcomes (21). We acknowledge that our model cannot make inferences as to whether the included risk markers are valid targets for therapy. A prospective trial targeting the multiple risk markers included in the PRE score is required to demonstrate this. Second, the model assumes that the predictive ability of short-term risk marker changes are independent of the drug or intervention. As such the model is potentially applicable to different interventions as long as all relevant risk marker changes are measured and included in the model, and risk marker-outcome relationships are not modified by treatment.

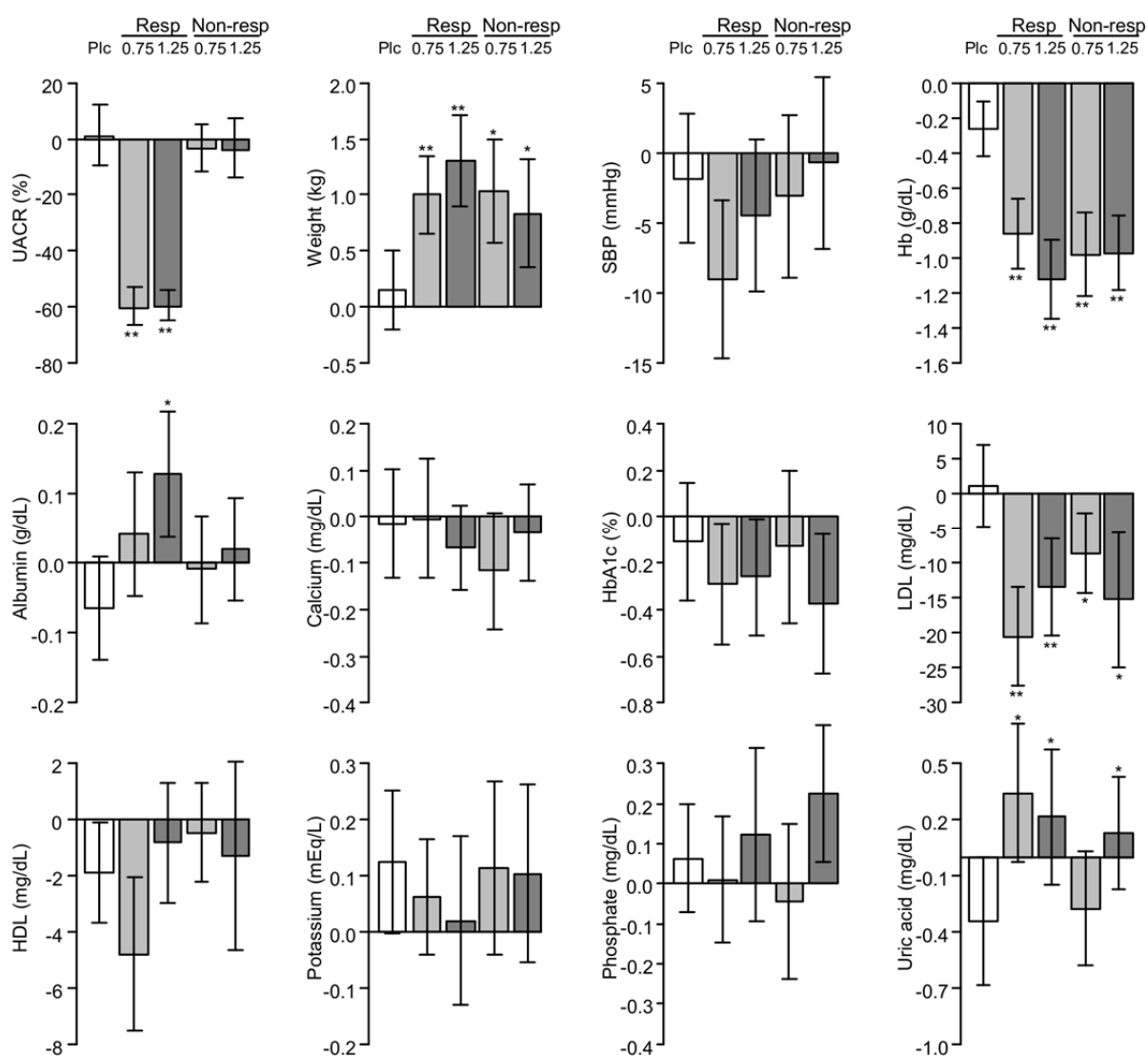
This study has limitations. Firstly, the follow-up duration in the RADAR/JAPAN trials was 12 weeks. The PRE score analysis assumes that initial week-12 changes in risk markers are sustained during long-term follow-up. However, long-term stability of risk marker changes depends on various factors including treatment compliance, use of co-medication, and progression of disease. Secondly, we recognize that the sample size of the RADAR/JAPAN trials was relatively small which is reflected by the large confidence intervals in predicted treatment effects. Thirdly, we used the changes in body weight as a proxy for fluid retention. However, it is well known that changes in body weight are variable and we did not use standardized techniques (e.g. same procedures and weighing scale in all patients) to measure body weight. This has introduced random variability and limited our ability to precisely predict the long-term effect on heart failure. Lastly, we cannot exclude that there were other relevant risk markers not measured in RADAR/JAPAN and therefore not accounted for in the PRE score.

In conclusion, based on short-term changes in risk markers, both atrasentan 0.75 mg/d and 1.25 mg/d are expected to decrease renal risk and slightly increase heart failure risk, the latter to a lesser extent with the low dose. Albuminuria responders to atrasentan (>30% reduction) are the major contributors to the predicted renal risk reductions. The predicted ratio of the renal risk reduction versus heart failure risk increase favors the atrasentan 0.75 mg/d dose. The ongoing hard outcome trial SONAR selects only patients with type 2 diabetes and nephropathy that respond (>30% albuminuria reduction) to atrasentan 0.75 mg/d, and will provide a more clear answer as to whether the PRE score predictions are accurate.

Acknowledgements

This work was performed as part of the Escher project (project nr. T6-503) within the framework of the Dutch Top Institute Pharma.

Supplement



Supplemental Figure S1. Overview of the risk marker changes for the responder and non-responder subset. For comparison, changes in risk markers in the overall placebo population are shown as well. Abbreviations: Plc, Placebo; Resp, Responder; Non-resp, Non-responder, SBP, systolic blood pressure, Hb, hemoglobin.

Supplementary Table S1.

<i>Renal endpoint</i>		
	Atrasentan 0.75mg/d	Atrasentan 1.25mg/d
Total population	-26% (-48 to -4)	-28% (-51 to -5)
UACR Responders	-51% (-73 to -28)	-45% (-68 to -22)
UACR non-responders	+1% (-22 to +23)	+1% (-33 to +36)
<i>Heart failure endpoint</i>		
	Atrasentan 0.75mg/d	Atrasentan 1.25mg/d
Total population	0% (-16 to +17)	+8% (-12 to +28)
UACR Responders	-8% (-26 to +10)	+6% (-16 to 28)
UACR non-responders	+8% (-11 to +27)	+12% (-16 to +39)
Predictions for the RADAR/JAPAN population (N=211) with imputed risk marker values in case of missing data. PRE scores are calculated for the total population, the responder subset and non-responder subset. Results are shown in mean (95% CI).		

References

1. Gregg EW, Li Y, Wang J, et al. Changes in diabetes-related complications in the united states, 1990–2010. *N Engl J Med* 2014; 370(16): 1514-1523.
2. Heerspink HJ, de Zeeuw D. The kidney in type II diabetes therapy. *Rev Diabet Stud* 2011; 8(3): 392-402.
3. Kohan DE, Pollock DM. Endothelin antagonists for diabetic and non-diabetic chronic kidney disease. *Br J Clin Pharmacol* 2013; 76(4): 573-579
4. Kohan DE, Pritchett Y, Molitch M, et al. Addition of atrasentan to renin-angiotensin system blockade reduces albuminuria in diabetic nephropathy. *J Am Soc Nephrol* 2011; 22(4): 763-772.
5. Kohan DE, Barton M. Endothelin and endothelin antagonists in chronic kidney disease. *Kidney Int* 2014; 86(5): 896-904.
6. Chandrashekar K, Juncos LA. Endothelin antagonists in diabetic nephropathy: Back to basics. *J Am Soc Nephrol* 2014; 25(5): 869-871.
7. Wenzel RR, Littke T, Kuranoff S, et al, for the SPP301 (Avosentan) Endothelin Antagonist Evaluation in Diabetic Nephropathy Study Investigators. Avosentan reduces albumin excretion in diabetics with macroalbuminuria. *J Am Soc Nephrol* 2009; 20(3): 655-664.
8. Weber MA, Black H, Bakris G, et al. A selective endothelin-receptor antagonist to reduce blood pressure in patients with treatment-resistant hypertension: A randomised, double-blind, placebo-controlled trial. *The Lancet* 2009; 374(9699): 1423-1431.
9. Dhaun N, Melville V, Blackwell S, et al. Endothelin-A receptor antagonism modifies cardiovascular risk factors in CKD. *J Am Soc Nephrol* 2013; 24(1): 31-36.
10. de Zeeuw D, Coll B, Andress D, et al. The endothelin antagonist atrasentan lowers residual albuminuria in patients with type 2 diabetic nephropathy. *J Am Soc Nephrol* 2014; 25(5): 1083-1093.
11. Mann JFE, Green D, Jamerson K, et al, for the ASCEND Study Group. Avosentan for overt diabetic nephropathy. *J Am Soc Nephrol* 2010; 21(3): 527-535.
12. Kalra PR, Moon JCC, Coats AJS. Do results of the ENABLE (endothelin antagonist bosentan for lowering cardiac events in heart failure) study spell the end for non-selective endothelin antagonism in heart failure? *Int J Cardiol* 2002; 85(2-3): 195-197.

13. Smink P, Hoekman J, Grobbee D, et al. A prediction of the renal and cardiovascular efficacy of aliskiren in ALTITUDE using short-term changes in multiple risk markers. *Eur J Prev Cardiol* 2013; 21(4): 434-441.
14. Smink PA, Miao Y, Eijkemans MJC, et al. The importance of short-term off-target effects in estimating the long-term renal and cardiovascular protection of angiotensin receptor blockers. *Clin Pharmacol Ther* 2014; 95(2): 208-215.
15. Heerspink HJL, Grobbee DE, de Zeeuw D. A novel approach for establishing cardiovascular drug efficacy. *Nat Rev Drug Discov* 2014; 13(12): 942.
16. Lambers Heerspink HJ, Gansevoort RT, Brenner BM, et al. Comparison of different measures of urinary protein excretion for prediction of renal events. *J Am Soc Nephrol* 2010; 21(8): 1355-1360.
17. Hoekman J, Lambers Heerspink HJ, Viberti G, Green D, Mann JFE, de Zeeuw D. Predictors of congestive heart failure after treatment with an endothelin receptor antagonist. *Clin J Am Soc Nephrol* 2014; 9(3): 490-498.
18. Heerspink HL, de Zeeuw D. Pharmacology: Defining the optimal dose of a new drug: A crucial decision. *Nat Rev Nephrol* 2009; 5(9): 498-500.
19. Temple R. Enrichment of clinical study populations. *Clin Pharmacol Ther* 2010; 88(6): 774-778.
20. Schievink B, de Zeeuw D, Parving H-H, et al. The renal protective effect of angiotensin receptor blockers depends on intra-individual response variation in multiple risk markers. *Br J Clin Pharmacol* 2015. doi: 10.1111/bcp.12655.
21. Moons K, Kengne A, Grobbee D, et al. Risk prediction models: II. External validation, model updating, and impact assessment. *Heart*. 2012; 98: 691-698.